

Screening of Protein Libraries by Anchor Less Display (ALD) and FACS

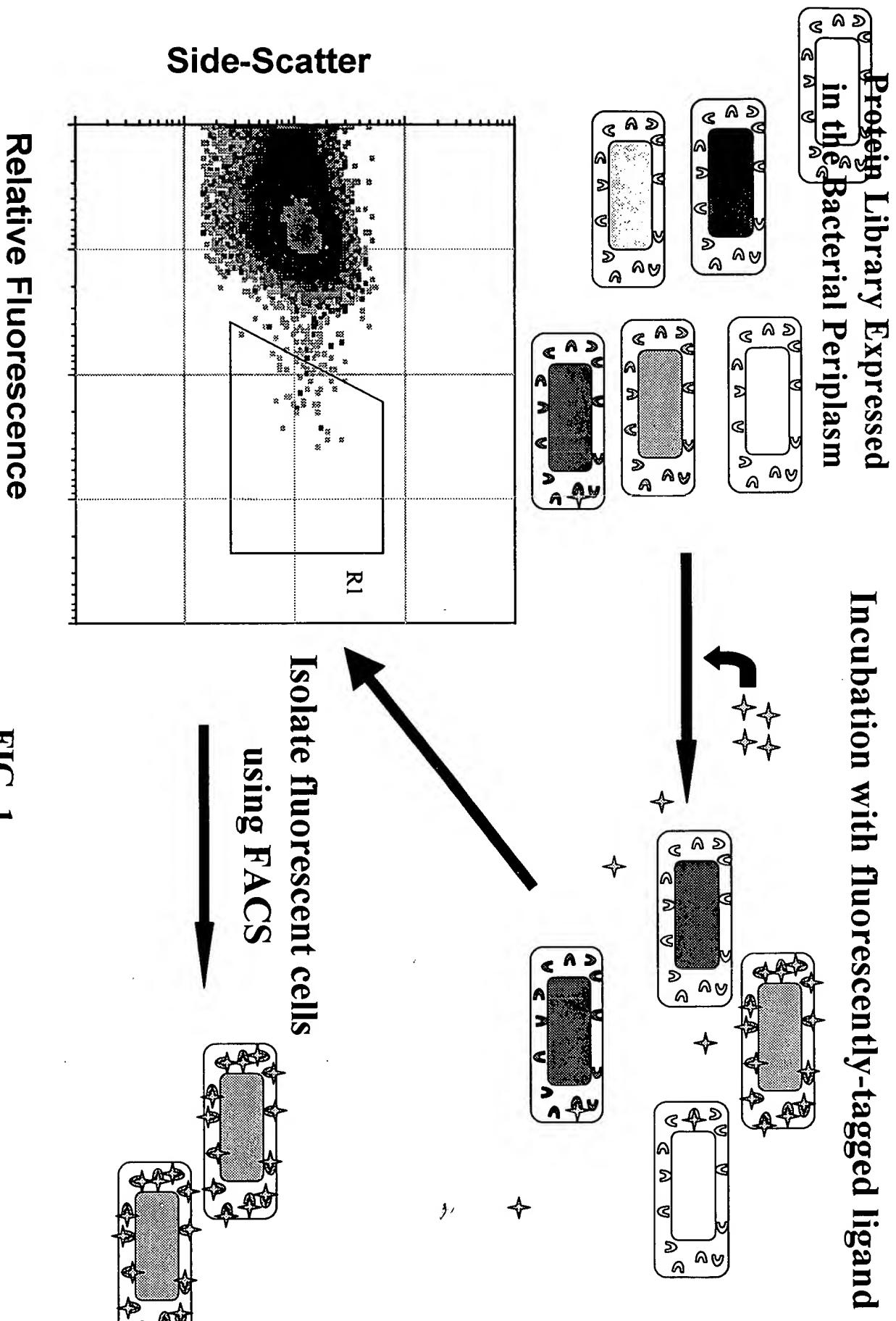
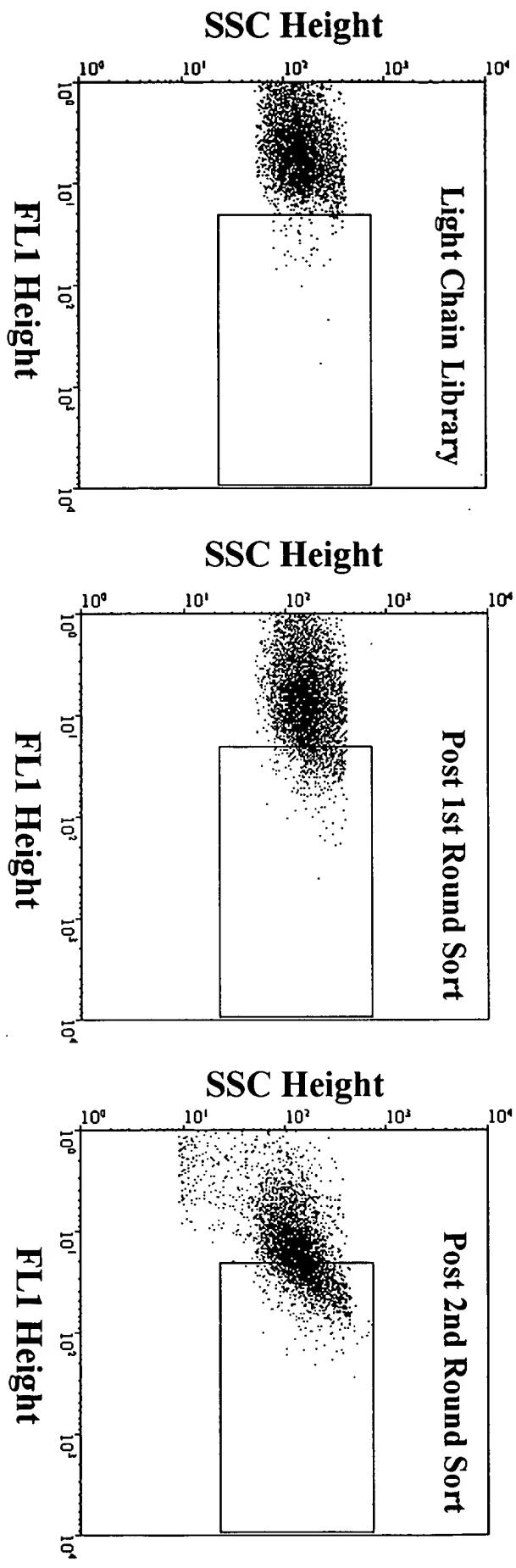


FIG. 1

Isolation of Affinity Improved Mutants by Two Rounds of Sorting



Size of the library = 2×10^6 transformants

FIG. 2

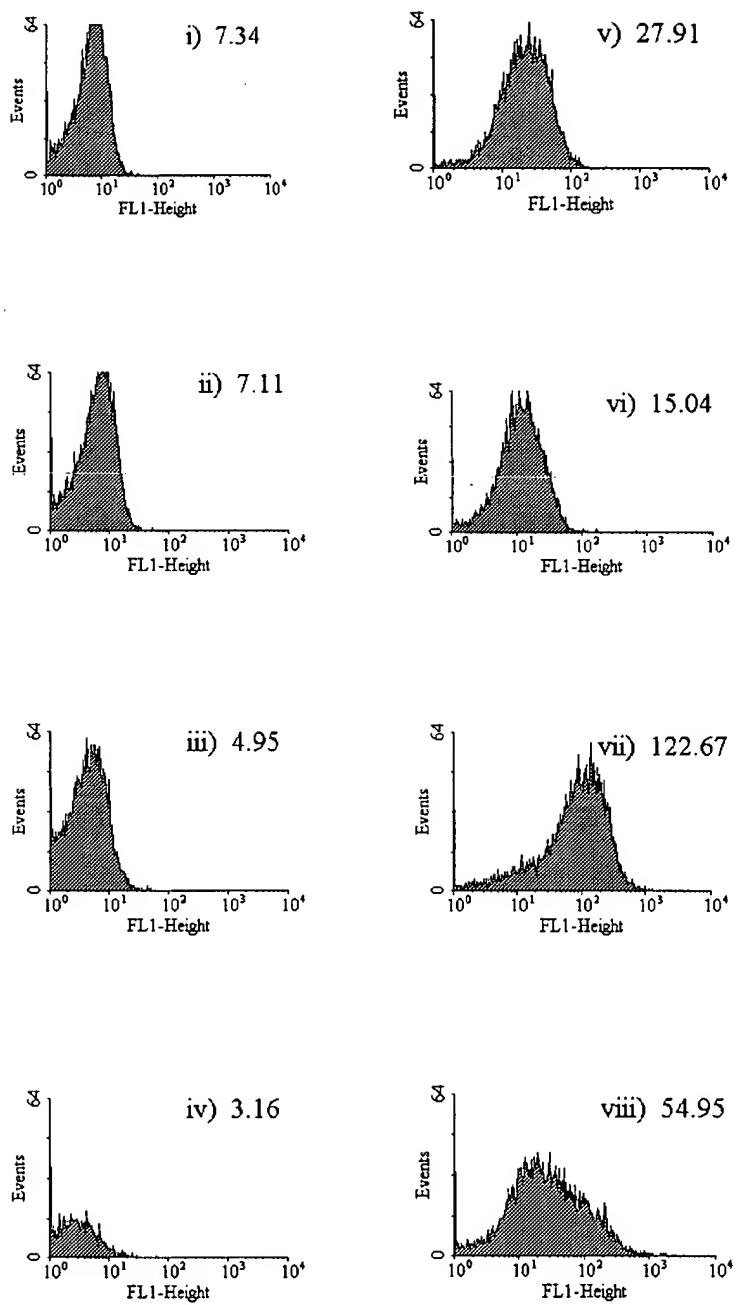


FIG. 3

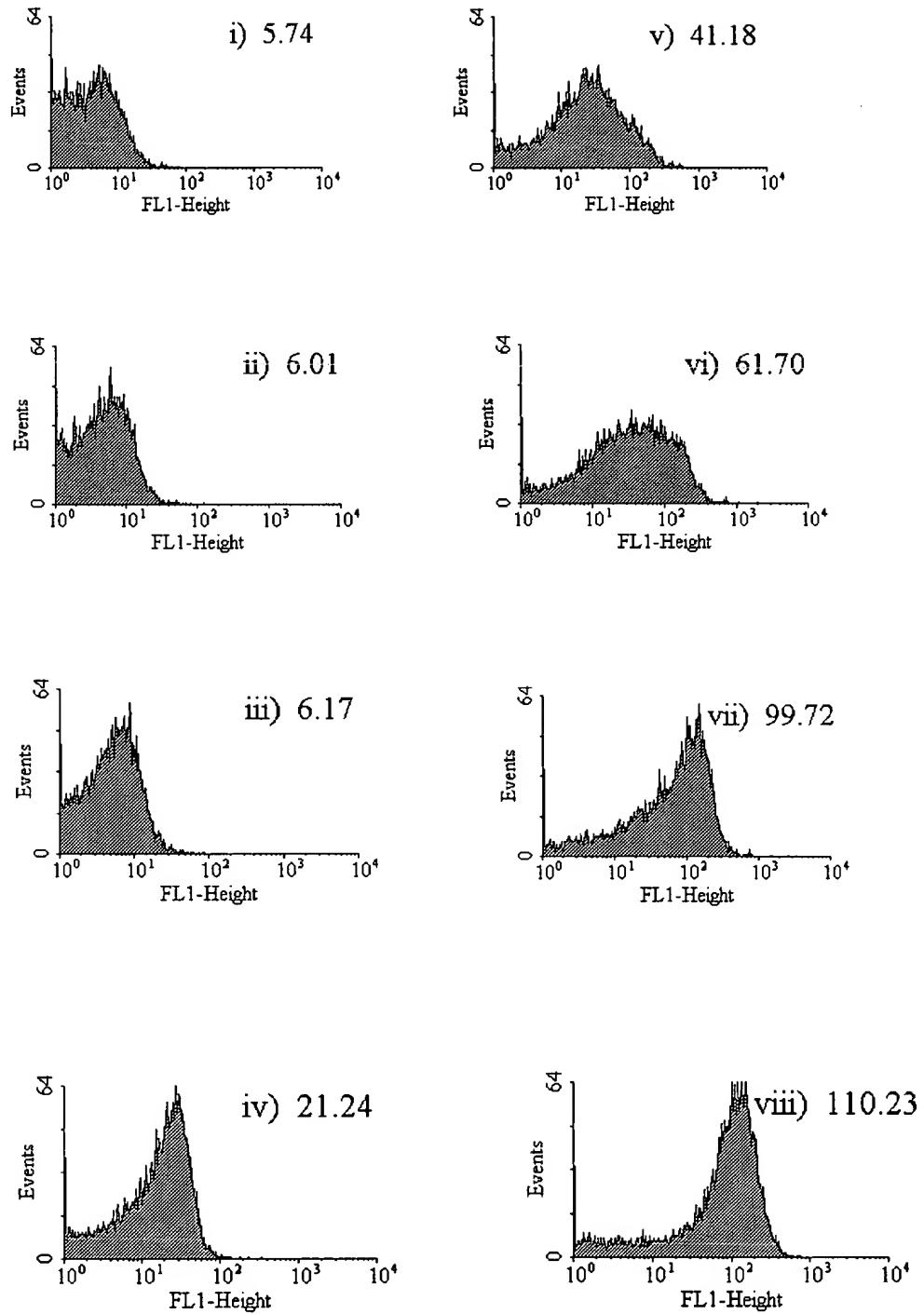


FIG. 4

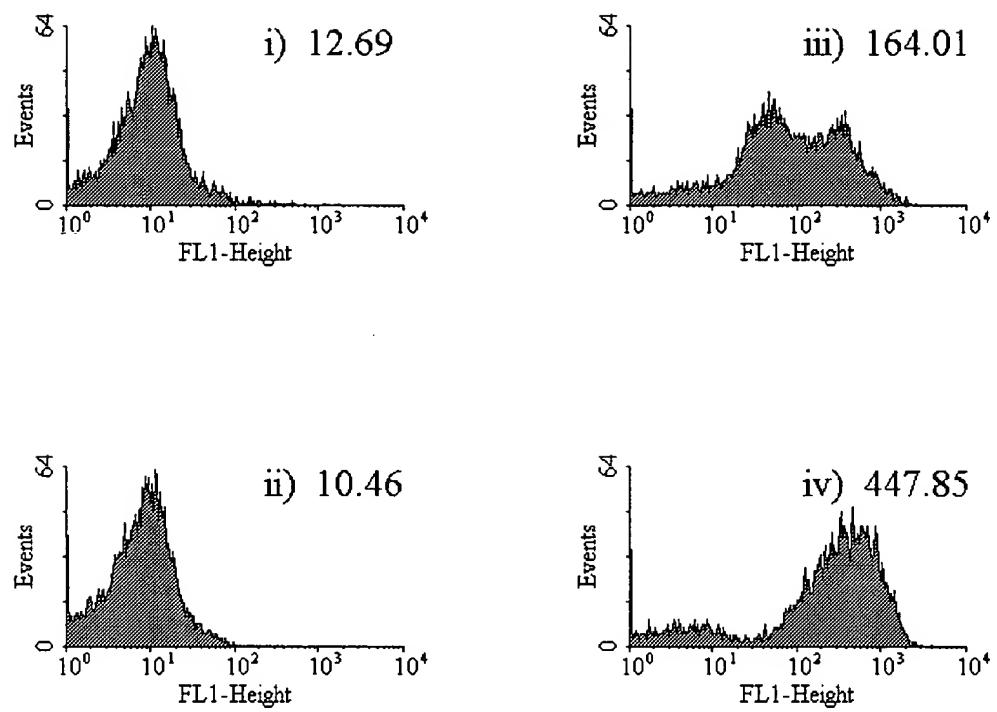


FIG. 5

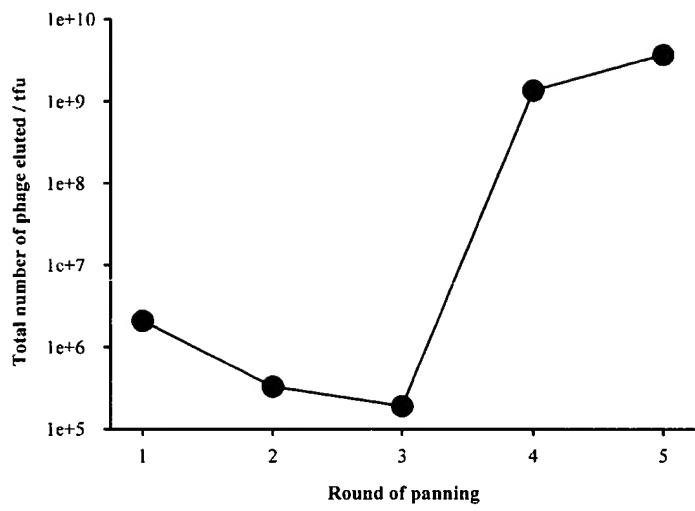
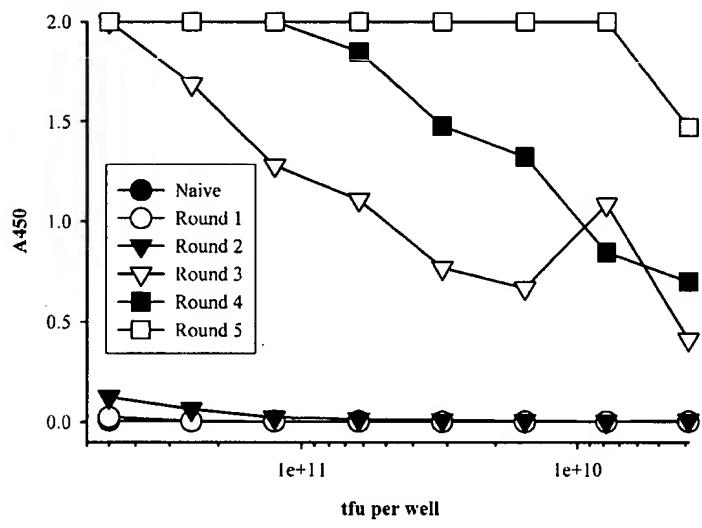


FIG. 6A

**FIG. 6B**

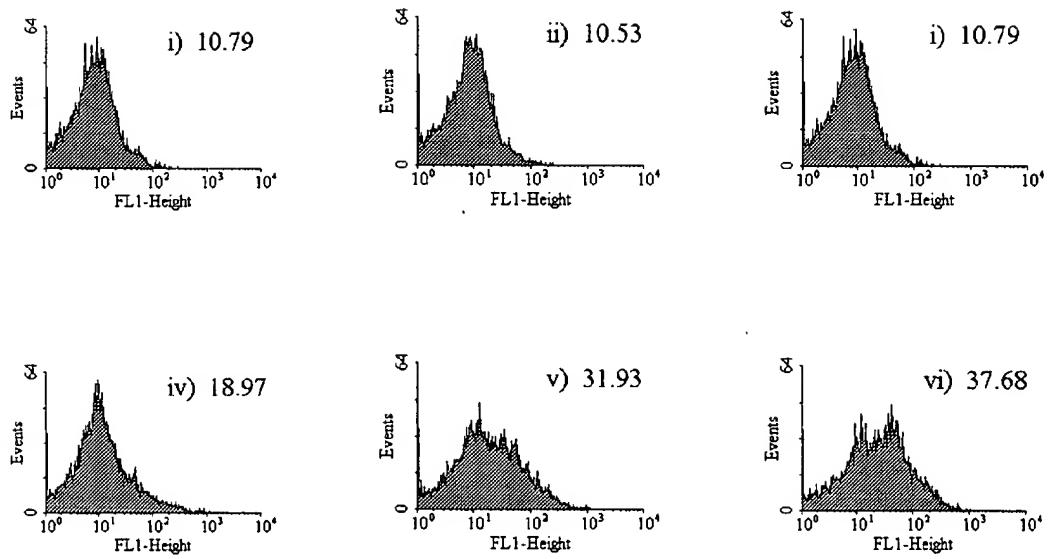


FIG. 6C

10

GlnValGlnLeuLeuGlnSerAlaAlaGluValLysLysProGlyGluSerLeuLys
CAGGTGCAGCTGTTGCAGTCTGCAGCAGAGGTGAAAAAGCCGGGGAGTCTCTGAAG
G AG GG G GCT GTC T A G C GA
ValGlu GlyGlyGlyLeuVal Gly Arg

20

30 CDR1

IleSerCysLysGlySerGlyTyrSerPheThrSerTyrTrpIleGlyTrpValArg
ATCTCCTGTAAGGGTTCTGGATACAGCTTACCAGCTACTGGATCGGCTGGGTGCGC
C GCA CC T C C GTGA AC GA A C
Leu AlaAla PheThr SerAsp TyrMetSer Ile

40

52a

GlnMetProGlyLysGlyLeuGluTrpMetGlyIleIleTyrProGlyAspSerAsp
CAGATGCCCGGGAAAGGCCTGGAGTGGATGGGATCATCTATCCTGGTACTCTGAT
GCT A G G G TTCATAC TAG AG A GTAGTACC
Ala ValSerTyr SerSerSerGly Thr

CDR2

70

ThrArgTyrSerProSerPheGlnGlyGlnValThrIleSerAlaAspLysSerIle
ACCAGATAACAGCCCGTCCTCCAAGGCCAGGTACCACATCTCAGCCGACAAGTCCATC
TATAC GCAGAC TG GA G GAT CAGG CG AG
IleTyr AlaAsp ValLys ArgPhe Arg AsnAlaLys

80

82a b c

90

SerThrAlaTyrLeuGlnTrpSerSerLeuLysAlaSerAspThrAlaValTyrTyr
AGCACCGCCTACCTGCAGTGGAGCAGCCTGGACACGGCCGTGTATTAC
A T ACTG T AAT A GA GA
AsnSerLeu MetAsn Arg Glu

CDR3

110

CysAlaArgAlaSerProSerGlyPheAspTyrTrpGlyGlnGlyThrLeuValThr
TGTGCAAGAGCTTCTCCTCGGGGTTGACTATTGGGGCCAAGGTACCCTGGTCACC
ACGGG TT C
ThrGlyPhePro
A G AT
ThrTyr

ValSerSer

GTCTCGAGT

FIG. 7A

20

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACC
GlnSerValLeuThrGlnProProSerAlaSerGlyThrProGlyGlnArgValThr

CDR1 31 a b

ATCTCTTGTCTGGAAGCAGCTCCAACATCGGAAGTAATTATGTATACTGGTACCAG
IleSerCysSerGlySerSerAsnIleGlySerAsnTyrValTyrTrpTyrGln

40

CDR2

CAGCTCCCAGGAACGGCCCCAAACTCCTCATCTATAGGAATAATCAGCGGCCCTCA
GlnLeuProGlyThrAlaProLysLeuLeuIleTyrArgAsnAsnGlnArgProSer

60

70

GGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATC
GlyValProAspArgPheSerGlySerLysSerGlyThrSerAlaSerLeuAlaIle

80

CDR3

AGTGGGCTCCGGTCCGAGGATGAGGCTGATTATTACTGTGCAGCATGGATGACAGC
SerGlyLeuArgSerGluAspGluAlaAspTyrTyrCysAlaAlaTrpAspAspSer

95 a b

100

CTGCGGGCTGTTGTATTGGCGGAGGGACCAAGCTGACCGTCCTA
LeuArgAlaValValPheGlyGlyGlyThrLysLeuThrValLeu

G G CC
GlyGlyPro
CTCG ---
ProArg---

FIG. 7 B

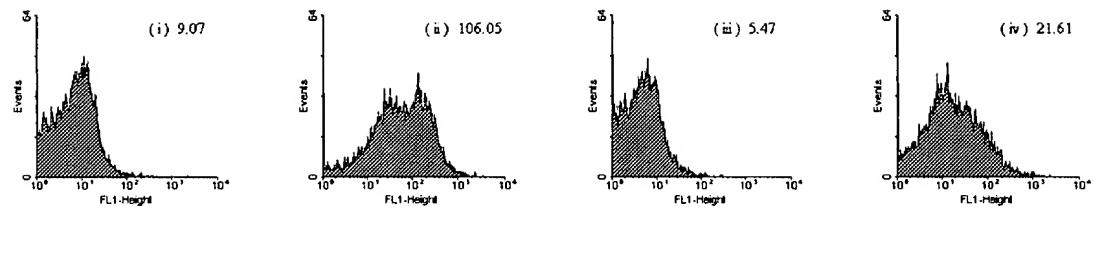


FIG. 8

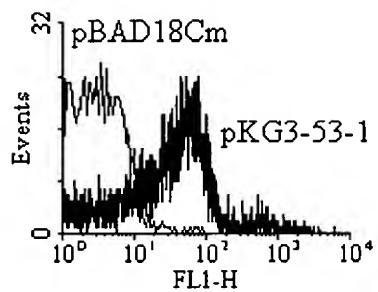


FIG. 9A

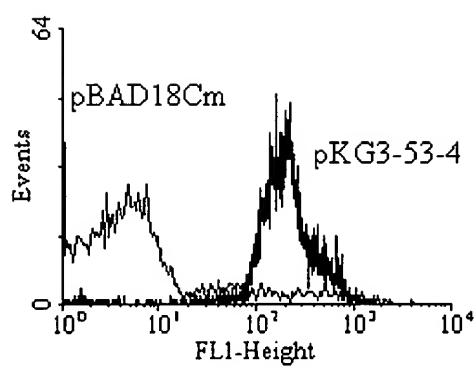


FIG. 9B